Effect Of Aegle Marmelos Correa On Blood Glucose Level In Normal And Alloxan-Induced Diabetic Albino Rats
S Baishnab, S Das

Citation

Abstract
The aim of the present study is to evaluate the action extract of Aegle marmelos correa on blood glucose level in normal and alloxan-induced diabetic albino rats. The aqueous extract (100mg/kg/d) was administered orally for four weeks to alloxan-induced diabetic rats. Blood glucose was estimated every week for four consecutive weeks. For evaluation of mechanism of action of test drug, glycogen estimation was done in liver, heart and skeletal muscle and effect on adrenaline-induced hyperglycemia was seen. The test drug significantly (p<0.01) reduced the rise in blood glucose induced by alloxan. The test drug produced significant (p<0.01) increase in liver glycogen and also significantly (p<0.01) reduced adrenaline-induced hyperglycaemia. No significant lowering of normal blood glucose was also found. Thus, the fruit of Aegle marmelos has significant antidiabetic activity.

INTRODUCTION
Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. A changing lifestyle in developing countries like India has enormously increased the statistical figures of diabetes mellitus. A survey depicts that 4% of adults in India suffered from DM in year 20000 and it is expected to increase by 6% by the year 2025.

Aegle marmelos correa is a tree belonging to the family Rutaceae, occurring throughout India, Sri Lanka, Pakistan, Bangladesh, Myanmar, Thailand and most Southeastern Asian countries. Aegle marmelos correa commonly known as Bael fruit is mildly astringent and used to cure dysentery, diarrhea, hepatitis, tuberculosis, dyspepsia and good for heart and brain. Phytochemical analysis of bael fruit has revealed the presence of alkaloids, coumarin, marmin, imperatorin, aegelin, tannins. The powdered bael fruit is used by the local people of Assam in the treatment of diabetes. Keeping this in view, the present study was aimed at evaluating the effect of Aegle marmelos correa on blood glucose level in normal and alloxan-induced diabetic albino rats with stress on evaluation of probable mechanism of antidiabetic action.

MATERIALS AND METHODS

PLANT MATERIAL AND EXTRACTION
The fruit pulp of Aegle marmelos were collected from the local market in Dibrugarh in the months of June to August. The plant material was authenticated by Dr S.S. Dhawan, Professor of food and nutrition department, HAU, Hisar, Haryana.

The fruit pulp of Aegle marmelos were manually separated from the outer seed shell, air dried, powdered (1600 g) and aqueous extracts were prepared using sufficient water by percolation method followed by steam evaporation. A final yield of 165 g of the extract was obtained.

ANIMALS
Healthy adult Wistar albino rats (Rattus norvegicus) weighing 200—250 grams each were used for the study. All the animals were taken care of under ethical consideration as per the guidelines of the CPCSEA with due approval from the Institutional Animal Ethical Committee (Registration no: 634/02/a/CPCSEA; dated 19/5/2002).

CHEMICALS USED
Crude powder of glibenclamide was obtained from Sun Pharma Ltd., Mumbai while alloxan monohydrate was purchased from Sigma Aldrich India, Bangalore. The glucose kit for blood glucose estimation was obtained from Sigma Diagnostic (India) Pvt. Ltd., Baroda.
EXPERIMENTAL DESIGN FOR ANTIDIABETIC STUDY

A total of thirty animals were equally divided into five groups with six animals in each group:

Group–A : Normal Control. Received normal saline, 2 ml/kg/d. Group–B: Normal Test. Received normal saline, 2 ml/kg/d. Group–C: Diabetic Control. Received normal saline, 2 ml/kg/d. Group–D: Diabetic Test. Received aqueous extract of Aegle marmelos (AEAM), 100 mg/kg/d. Group–E: Diabetic Standard. Received glibenclamide, 2.5 mg/kg/d.

The above drugs were administered orally, once daily, for four weeks.

INDUCTION OF DIABETES

Leaving aside six rats for Normal Control Group, 24 rats were induced diabetes by a single intraperitoneal injection of alloxan monohydrate in the dose of 150 mg/kg body weight. The fasting blood glucose was determined after 72 hours. 18 rats showing blood glucose level greater than 200 mg/100 ml were taken for the study.

Blood glucose was estimated every week for four consecutive weeks. Blood glucose estimation was done by glucose oxidase method.

PROBABLE MECHANISM OF ANTIDIABETIC ACTION

GLYCOGEN ESTIMATION OF LIVER, SKELETAL MUSCLE AND CARDIAC MUSCLE.

Out of 30 rats, 24 rats were induced diabetes by alloxan monohydrate (150 mg/kg body weight) intraperitoneally and 18 rats with blood glucose level greater than 200 mg/100ml were taken after 72 hours of diabetes induction. All the rats were kept fasting for 18 hours before the experiment. The rats were divided into four groups with six animals in each, as before.

Group–A: Normal Control. Received normal saline, 2 ml/kg/d. Group–B: Diabetic Control. Received normal saline, 2 ml/kg/d and alloxan. Group–C: Diabetic Test. Received AEAM, 100 mg/kg/d and alloxan. Group–D: Diabetic Standard. Received glibenclamide, 0.5 mg/kg/d and alloxan.

After two hours of administration of above drugs. The animals were killed by decapitation. The liver, leg muscle and heart tissues were taken out with care and their glycogen content was estimated by use of Anthrone reagent.

EFFECT ON ADRENALINE-INDUCED HYPERGLYCEMIA

The rats were divided into three groups with six animals in each as before.

Group–A : Normal Control. Received normal saline, 2 ml/kg/d. Group–B: Test Drug. Received AEAM, 100 mg/kg/d. Group–C: Standard Drug. Received glibenclamide, 0.5 mg/kg/d.

The above drugs were administered orally after drawing fasting blood samples. Adrenaline hydrochloride 100 µg was administered intraperitoneally to all the rats one hour after drug administration. Blood samples were again collected half an hour.

STATISTICAL ANALYSIS

The data was statistically analysed using One-way ANOVA followed by Dunnett’s multiple comparison test. Values of p < 0.01 were considered significant.

RESULTS

EFFECT ON BLOOD GLUCOSE LEVEL

The data was statistically analysed using One-way ANOVA followed by Dunnett’s multiple comparison test.

Normal Rats: No significant (p > 0.01) difference of blood glucose level was found in Normal Control Group and Normal test Group after four weeks of drug administration.

Diabetic Rats: On repeated administration of the extract and glibenclamide for four weeks, a significant (p < 0.01) decrease in blood glucose was found in Diabetic Test Group and Diabetic Standard Group respectively as compared to Diabetic Control Group which showed a significant p < 0.01) rise in blood glucose as compared to Normal Control Group. However, both the drugs failed to restore the blood glucose level to that of the Normal Control Group (Table–1).

Values are expressed as Mean ± SEM; n=6 rats in each group. One-way ANOVA followed by Dunnett’s multiple comparison tests was done. *p<0.01 when compared to Normal Control Group. "p<0.01 when compared to Diabetic Control Group.
Effect Of Aegle Marmelos Correa On Blood Glucose Level In Normal And Alloxan-Induced Diabetic Albino Rats

Figure 1

Table-1: Effect of AEAM on blood glucose level of alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Glucose Level (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 72 Hours</td>
</tr>
<tr>
<td>Normal Control</td>
<td>52.1 ± 0.5</td>
</tr>
<tr>
<td>Normal Test</td>
<td>52.1 ± 0.5</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>92.1 ± 0.5</td>
</tr>
<tr>
<td>Diabetic Test</td>
<td>92.1 ± 0.5</td>
</tr>
<tr>
<td>Diabetic Standard</td>
<td>92.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM; n=6 rats in each group. One-way ANOVA followed by Dunnett’s multiple comparison tests was done. * p <0.01 when compared to Diabetic Control Group.

Figure 2

Table-2: Effect of AEAM on glycogen concentration in liver, skeletal muscle and cardiac muscle

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Glycogen Concentration (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Normal Control</td>
<td>50 ± 0.5</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>20 ± 0.5</td>
</tr>
<tr>
<td>Diabetic Test</td>
<td>72 ± 0.95</td>
</tr>
<tr>
<td>Diabetic Standard</td>
<td>25 ± 1.5</td>
</tr>
</tbody>
</table>

One way ANOVA (F) 742, Degree of Freedom 2, 20, *p* value > 0.01

Figure 3

Table-3: Effect of AEAM on adrenaline-induced hyperglycemia in albino rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Blood Glucose Level (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%0 Hour (Fasting)</td>
</tr>
<tr>
<td>Control</td>
<td>100 ± 1.1</td>
</tr>
<tr>
<td>Test (100 mg/kg)</td>
<td>150 ± 1.5</td>
</tr>
<tr>
<td>Standard</td>
<td>150 ± 1.2</td>
</tr>
</tbody>
</table>

One way ANOVA (F) 1.8, Degree of Freedom 2, 16, *p* value > 0.01

DISCUSSION

From the study, it was seen that AEAM significantly (p < 0.01) lowered the blood glucose level in diabetic rats. However, AEAM does not lowered the normal blood glucose level in normal test group when compared to normal control group. The anti-hyperglycemic action of AEAM may be attributed to the insulin-like effects of the constituents of the fruit pulp of Aegle Marmelos. Raised blood glucose level is the principal stimulus for insulin secretion. However, the probability of an insulin releasing action of the constituents of Aegle Marmelos cannot be ruled out.

Alloxan, a β-cytotoxic agent, rapidly and selectively accumulates in pancreatic β-cells and causes β-cell death and apoptosis by generation of reactive oxygen species (ROS), superoxide radicals and hydrogen peroxide. β-cell death causes hyperglycemia due to insulin deficiency which further aggravates the oxidative stress induced by alloxan.
might be attributed to the presence in it of Coumarins which potentiate the insulin secretion from existing beta cells of the islets of langerhans. Antioxidative parameters like reduced glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase have shown a dose related increase in their level/activity and decrease in lipid peroxidation following the treatment with fruit extract. The antioxidant activity might be due to the presence of phenolic compounds such as flavonoids. Furthermore, previous studies have reported the protective action of flavonoids against oxidative stress induced cellular damage and also the ability of flavonoids to regenerate β-cells.

Insulin is a potent activator of the enzyme glycogen synthase while inhibiting the enzyme glycogen phosphorylase responsible for glycogenolysis in liver and muscle. Insulin deficiency in diabetes, as such, results in reduced concentrations of glycogen in liver and muscle. AEAM caused an increase in glycogen concentration of the liver probably by stimulating the enzymes glycogen synthase and hexokinase, both of which contribute to increase glycogen synthesis. The increase in liver glycogen may also have been brought about by inhibition of the enzyme glucose-6-phosphatase leading to accumulation of glucose-6-phosphate, which allosterically inhibited the enzyme glycogen phosphorylase. Diminished phosphatidylinositol 3-kinase (PI-3K) activation in diabetes as a result of insulin deficiency has been reported to be associated with impaired skeletal muscle glycogen synthase enzyme. AEAM due to the insulin-like action of its ingredients probably increased PI-3K activation leading to stimulation of muscle glycogen synthase. The increased concentration of glycogen in skeletal and cardiac muscle also might be due to increased expression and translocation of GLUT-4 glucose transporters as a result of increased PI-3K activation, leading to increased peripheral uptake of glucose.

Adrenaline produces hyperglycemia by inhibiting insulin release, stimulating glycogenolysis in muscle and thus providing substrate in the form of lactate for hepatic gluconeogenesis, stimulating glucagon secretion and stimulating ACTH secretion which, in turn, stimulates glucocorticoid secretion from the adrenal cortex. It has also been reported that adrenaline produces hyperglycemia by increasing glucose uptake from both the large and small intestine. The test drug significantly (p<0.01) reduced the adrenaline induced hyperglycemia probably by inhibiting adrenaline induced stimulation of β receptors in β-cells of pancreas and thus promoting further insulin release.

CONCLUSION

Thus, the hypoglycemic and antidiabetic effect of AEAM may be partly due to its positive effect on glycogen synthesis in liver, skeletal muscle and heart muscle due to the insulin-like action of its constituents, and partly due to the stimulatory action on insulin release by blocking the β receptors in β-cells of pancreas. However, further studies to isolate the active principle of Aegle Marmelos responsible for hypoglycemia, together with studies on serum insulin assay to confirm its insulin releasing action have to be undertaken.

References


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